Studying osm1/ syp61 gene in arabidopsis under drought stress

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Abstract:

Drought is a major abiotic stress that causes a reduction in plant growth and yields of agricultural crops. Naturally, Plants differ in their susceptibility to drought adaptation and tolerance and research confirmed that many genes and transcription factors are involved in the response to water deficit. I tested Arabidopsis OSM1 (for osmotic stress-sensitive mutant 1) (At1g28490) gene- which is similar to SYP61 (SYNTAXIN OF PLANTS 61) -under drought stress for the first time in plants grown on soil. The investigations included wild type plants and osm 1 mutant line (SALK_107167) subjected to drought. The sequence analysis showed that the T-DNA in this mutant is inserted in the promoter and in the correct site as reported by the database but showed smaller product size than the expected confirming missing of 86 bases at the left border of the T-DNA. Genotyping investigations showed that the lines segregated out according to the mendelian bases. The mutant plants were significantly more sensitive to wilting when grown with limited soil moisture compared to wild-type plants but plant survival was not affected. These results indicate that this gene may be involved in drought stress response and wider experimentation is needed to include transcript accumulations under such kind of stress in parallel with the use of a mutant that carries a T-DNA within the gene. .

Keywords: OSM1, SYP61, T-DNA, drought stress, mutant line, Arabidopsis.

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Introduction:

Drought is a major abiotic stress that causes crop loss all over the globe and results in reducing yields. This stress is likely to become more severe with the increase of global climate change [3, 19] and the development of crop plants better adapted to water-limited environments is a priority for sustainable agriculture.

The availability of water is the most significant limiting factor which affects growth and productivity of plant crops; and the distribution of plant species [21,22, 27]. Plants differ in their susceptibility to drought and the difference has been referred to the gene expression and genomic content between species [5].

Generally, water stress causes various physiological and biochemical responses in plants. When a plant or cell is exposed to dehydration, it loses water and a reduction in turgor pressure takes place. Under these circumstances, plants accumulate abscisic acid (ABA) which has control on many adaptive responses. As stress continues to develop, ABA increases, leading to earlier stomatal closure and a decrease in photosynthesis. With additional stress, respiration decreases and proline, an amino acid, increases.

The dissection of moderate drought (MDr) stress responses using a time-course analysis of biochemical, physiological, and molecular processes revealed early accumulation of ABA and induction of associated signalling genes, conformable with a decrease in stomatal conductance as an early avoidance response to drought stress. This process is accompanied by a peak in the expression of expansin genes involved in cell wall expansion leading to cell wall adjustment; a process toward drought stress acclimation [9, 10]. Also, it is confirmed in *Arabidopsis thaliana* guard cells that members of the AtVAMP71 complex (a member of SNARE family) play an important role in the proper reactive oxygen species (ROS) localization, regulating stomatal closure after ABA treatment [10]. The syntaxins family of soluble N-ethylmaleimide sensitive factor adaptor protein receptors (SNAREs) are a large group of proteins which have a function in

the fusion of transport vesicles to target membranes in eukaryotes [25, 29]. The OSM1 gene which is related most closely to mammalian syntaxins 6 and 10 (members of SNARE), was implicated in osmotic stress tolerance [30]. It is also involved in the stomatal response regulated by ABA [11].

In general, many genes have been tested for their function under different abiotic stresses and found to be upregulated upon exposing to one or more of the abiotic stresses. Related to my work, OSM1/SYP61 (Syntaxin of Plants 61) gene (which is related most closely to mammalian syntaxins 6 and 10 (members of SNARE), was found to have a role in response to osmotic stress and in the ABA regulation of stomatal responses [30] using a mutant whose T-DNA is in the coding region, and in response to cold stress [8] upon using a mutant carrying a T-DNA in the promoter. However, it has been found that the T-DNA being inserted in the promoter of OSM1 gene has led to higher gene expression in the mutant with better plant survival compared to the wild type plants [8]. These results are the outcome of two different experiments using two different mutants for this gene and they confirm the involvement of this gene in different abiotic stresses in Arabidopsis.

Studying abiotic stresses in Arabidopsis opens the way to modify genetically plant crops to gain tolerance against these reverse environmental conditions. A small weedy dicotyledonous plant, *Arabidopsis thaliana* has been used as a model to investigate and control stress in plants. Although Arabidopsis is not an economically important plant, it has become the focus of intensive genetic, physiological, biochemical, and molecular studies for many years. It is a member of the brassicaceae which includes many of the economic plant crops such as cabbage, broccoli, canola, turnip and radish. Arabidopsis as a model plant has many advantages for molecular and genetic studies. It has short life cycle, large number of progenies, and relatively small manipulable genome, whose sequence is now available, that can be manipulated

through genetic engineering more easily and rapidly than any other plant genome [31].

The T-DNA mutagenesis has become a very crucial strategy in molecular and biotechnological studies. The insertional mutagenesis techniques including transposone- and T-DNA mutagenesis, are key resources for systematic identification of gene function in Arabidopsis [1, 23]. So, it is a way to disrupt gene function by integrating a foreign DNA into the host sequence providing knockout mutants and also may lead to gene upregulation when it is inserted in the promoter [12].

The segregation analysis of the Arabidopsis insertional mutants indicates that, usually, there are more than one insertion in a line and inserting a T-DNA into a gene can lead to loss or gain of function [24]. The use of T-DNA as insertinoal mutagen has advantages. The advantage of T-DNA over classical mutagens is that the Arabidopsis sequences flanking the insertion site can be isolated easily. This simplifies the identification of genes corresponding to interesting mutants. The T-DNA is physically and chemically stable for many generations. Also, the sequence which flanks the insertion site can be easily isolated which makes the identification of the gene of interest so simple [24].

In this experiment, a T-DNA*osm1*mutant (SALK_107167) line of OSM1 gene in *Arabidopsis thaliana* was used under drought conditions comparing to the un-mutagenized plants. This experiment was designed to include two lines (homozygous and wild type) with five replicates with each replicate including three plants to be tested under water deficiency in soil.

Materials And Methods:

Growing Arabidopsis plants:

The experiments were conducted in controlled growth and cold rooms in the Horticulture Department, Faculty of Agriculture, University of Tripoli. Plants of *Arabidopsis thaliana* homozygous and wild type lines of OSM1 gene (At1g28490) were grown in separate pots. T5 seeds, provided by Nottingham Arabidopsis Stock

Centre (NASC) ((http://arabidopsis.info/), were used for this study. Seeds of the Salk line (SALK _107167) of OSM1were sown on peat moss produced by Floragard Company. After sowing, pots were covered with cling film and put in a cold room (±4ºC and continuous light) for 4-5 days. Pots were, then, transferred to a normal conditioned growth room with 16-22ºC. Short-day conditions (10hrs day - 14hrs night) were provided for vegetation, with light density about 170 µmol m^{-2} s^{-1} (400-700nm), and 50-75% relative humidity. Generally, plants used for this study were grown for a period recognized between stages 3-6 according to Boyes et al. [3].

Performing drought stresses:

After regular irrigation, water was withheld for 5 days then plants were watered again and then plants were exposed to drought stress for 22 days. The plant survival was recorded after 3 and 11 days from breaking drought stress. Images were taken before and after carrying out drought stress.

DNA isolation:

Genomic DNA of leaf samples was isolated (100-200mg leaf powder) using modified Edwards' method [7]. Leaf samples were collected and put in autoclaved Eppendorf tubes and put immediately in liquid nitrogen and then kept in a -20°C freezer for later use. Autoclaved mortars and pestles were used to grind leaf samples using small amount of liquid nitrogen twice to collect fine powder. The collected leaf powder was put in new autoclaved micro centrifuge tubes and 400 µl of Edward buffer (200mM Tris-Cl pH 7.5, 250 mMNaCl, 250 MM EDTA pH 8.0, 0.5% w/v SDS) was added. Then, mixtures were vortexed for 5-10 seconds and left for 2 minutes at room temperature. The mixture was centrifuged at maximum speed (13000 rpm) for 1 minute using (Hettich MIKROLITER) centrifuge. Supernatant (upper phase) (300 µl) was transferred to new micro centrifuge tubes and 300µl isopropanol (#I9516/I90764, Sigma Aldrich) was added. Gentle mixing was done and samples were left at room temperature for 2 minutes for well mixing. Samples were centrifuged at 12000 rpm

speed for 5 minutes using refrigerated micro centrifuge (5415, Eppendorf). The supernatant was removed, and DNA precipitated as pellets which were seen at the bottom of tubes. After the collection of DNA, samples were air dried for 2-4 minutes to clear off the isopropanol. To dissolve the DNA, pellets were re-suspended in 100µl dH_2O and stored at -20ºC for later use for PCR amplification.

DNA quantification:

All DNA samples for PCR were quantified using a UNICAM UV2 UV/Vis spectrometer and the absorbance at certain wave length (260, 280, and 320nm) was measured. 2 µl of DNA were added to 498µl of DEPC-treated water (0.5ml of diethylpyrocarbonate added to 1000ml deionized water) and also DEPC-treated water was used as a blank. The absorbance was measured for the amount of the nucleic acid for total volume 500µl after transferring the solution to a quartz cuvette for reading the absorbance. The nucleic acid ratio was measured and was found to range between 1.7 and 2.0.

PCR reaction:

For amplifying target DNA sequence, PCR reactions were performed using a thermo cycler (Px2 Thermal cycler, Thermo Electron Corporation) PCR machine. A certain PCR program was used to run the reactions using *Taq* DNA kit (#BIO21070, BiolineLbs Ltd) which included; 10 x buffer, $MgCl_2$, dNTP, and TAQ polymerase. Forward and reverse primers were diluted 10 times and used after preparing the primers stock solutions using DEPC water to obtain 10 pmol/µl. All solutions and template genomic DNA were thawed and vortexed gently and centrifuged briefly before preparing the master mix. The master mix was prepared with 10% extra amount to ensure the required volume of each reaction. The master mix was prepared from NH_4 buffer, $MgCl_2$, dNTP, specific primers, and Bio *Taq* DNA polymerase. Certain volume of the master mix was pipetted into each 0.2ml PCR thermal tube and then, dH_2O and DNA were added. The total

volume of each PCR reaction was 20µl and reactions were run according to a specific PCR program and then, PCR products were stored in a -20ºC deep freezer for later use.

Agarose gel electrophoresis for DNA:

Many equipments and chemicals were used for running gel electrophoresis. These include an electrophoresis chamber and power supply (Pharmacia GNA 200), gel casting trays with different sizes, well combs, electrophoresis Tris Borate EDTA buffer (TBE buffer # T4415, Sigma Aldrich), Ethidium bromide (EB) (for visualization), agarose (#300-300, Biogene), 5x DNA loading buffer 'blue dye'(#BIO37045, Bioline labs Ltd), Hyperladder IV quantitative (#BIO33029,BIioline), and gel documentation chamber (BIORAD).TBE buffer was added to Hi-pure Low EEO agarose powder (1%) and boiled until the agarose completely melted. This solution was allowed to cool down to (45-55°C) at room temperature and 10mg/ml ethidium bromide was added and the solution was mixed by swirling and poured into a taped casting tray (gel former) containing plastic well combs and allowed to solidify at room temperature. After proper solidification, tapes and combs were removed carefully and 1µg DNA (5-10µl) mixed with the loading buffer (1µl) was loaded (dry loading) into gel wells. Gel tray was then placed into the gel electrophoresis tank which was filled with electrolyte solution (TBE buffer) up to the level of the gel and covered with the lid. Gel electrophoresis was allowed to run in a fume cupboard after placing power leads in the apparatus and a potential difference of 80-90 voltage was applied. The gel was left to run in a fume cupboard for 1:30-2 hours until DNA migrated to a proper distance and DNA fragments got stained with ethidium bromide (EB) and then, visualized by UV tranilluminator using gel documentation chamber attached to a computer immediately after running the gel before DNA diffuses within the gel according to Bowen [2]. The quality of the DNA was assessed by observing a clear single illuminated band and smearing was considered sample degradation.

Purification of DNA PCR products:

For PCR products purification, a QIAquick PCR purification kit (50) (#28104) was ordered from Qiagene and used according to the instructions stated. A volume of 250 µl of PB buffer was mixed with 50µl of PCR product sample in an eppendorf tube. The mixture was poured into QIAquick spin column and centrifuged for 60s. The collected solutions were discarded and tubes were washed with 375µl PE buffer and centrifuged again for 60s. The collected liquid was discarded and centrifugation was done for 1 minute to remove any ethanol residues. The spin column was placed into autoclaved eppendorf tube and centrifuged for 1 minute, after adding 25 and then 15µl of dH_2O, to collect DNA bound to the column. Finally, the collected solution was transferred to a clean eppendorf tube and stored in a -20°C freezer for further use for DNA sequencing using 3x diluted primers for the target gene [32].

Sequencing PCR products and DNA alignment:

The purified PCR products of DNA were sent for sequencing along with diluted primers to the Institute for Research on Environment and Sustainability (IRES) sequencing services at Newcastle University,UK. The resulted sequences were aligned and compared with the known sequences from the database for the target gene using the following software and URLs:

Clustalwhttp://www.ebi.ac.uk/Tools/clustalw2/index.html, [6,17].

BioEdithttp://www.mbio.ncsu.edu/bioedit/bioedit.html, [16].

Finch TV http://www.geospiza.com/Products/finchtv.shtml.

Results And Disscussion:

Molecular analysis of osm1 mutant (SALK_107167):

The T-DNA of *osm1* (At1g28490) mutant appeared in the promoter region (upstream) of the gene on chromosome 1 (F3M18.7) as shown in Figure 1. PCR was performed on the

genomic DNA to identify wild type, heterozygous, and homozygous lines using specific primers. Amplifying the DNA from homozygous lines indicated that the insert is in the correct site as reported by the database and showed smaller product size than the expected. Sequence results confirmed that 86 bases were deleted at the left border of the T-DNA (Figures 2,4). This explains the small product size resulting from the amplification of the junction of *OSM1*::T-DNA using forward(F_2)[3' CAACCCTATCTCGGGCTATTC 5'] and reverse (R)[5' ATTGGGCTCTGTGGTCTCTT 3'] primers comparing to the expected one (Data not shown). Similar results were collected by Zhu et al. in Ref. [29] when they found slightly smaller product size in the mutant. Using F_2R primer pairs with wild type lines did not yield products (Figure 2). In contrast, the appropriate product size 367bp was confirmed from the genomic DNA template of wild type using forward (F_1) [3' AGATTTCCGGTGCACAGAAG 5'] and reverse (R)[5' ATTGGGCTCT GTGGTCTCTT 3'] primers (Figures 2,5).To confirm the lack of insert in the wild plants, forward (F) [5' TTGCTTTGAAGACGTGGTTG 3'] and reverse (R) [3' CAGCAGGTCTCATCAAGACG 5'] primer pair was used to amplify 665bp of T-DNA only. All tested wild type lines did not show any band (Figure 3) indicating the absence of T-DNA at least from the *OSM1* gene and in the nearest region of the genome.

***OSM1* (At1g28490, SALK_107167, N607167)**

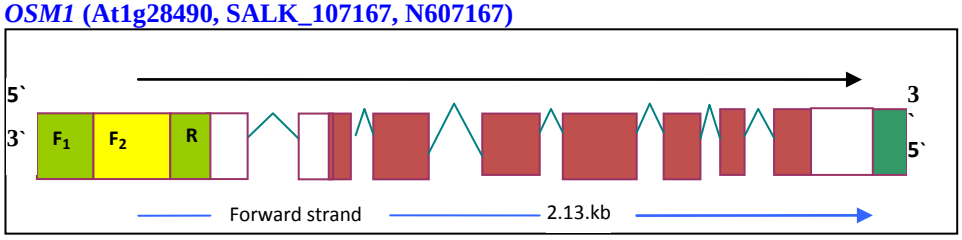

Figure 1: An *AtOSM1* gene model diagram showing different regions and the site of the forward and the reverse primers amplifying either the host sequence of the target genes (for the wild type) and the host sequence with T-DNA (for the mutant); upstream sequence (light green), downstream (dark green), T-DNA (yellow), exons (red), introns (green angled lines), un-translated region (empty boxes), F (forward primer), and R (reverse primer). Modified from (http://arabidopsis.info/).

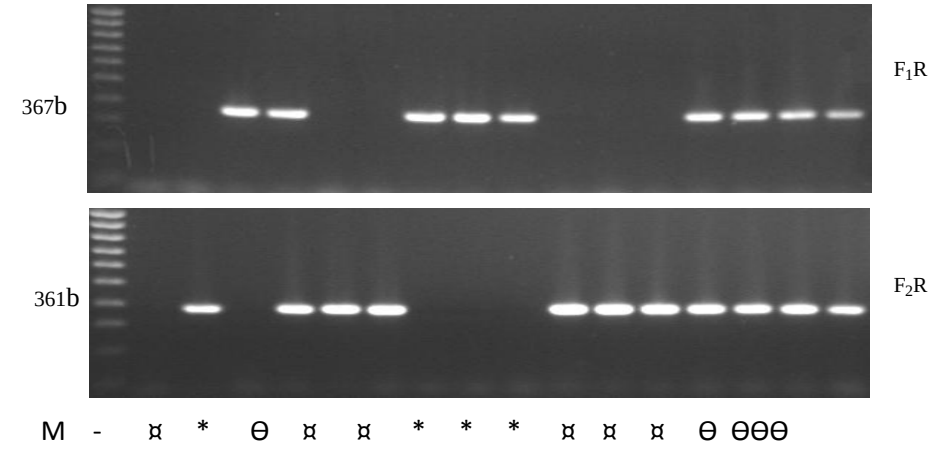

Figure 2: A Sample of identifying *OSM1* genotype using specific primers F1R for the wild type and F2R for the insert, (*) wild type, (¤) homozygous, and (Θ) heterozygous. The marker (M) used is 1kb and each band indicates 100 bp.Negative control = no DNA (-).

Figure 3: A gel electrophoresis sample of PCR using FR primers to amplify only the T-DNA in *OSM1* wild type lines (*). A Heterozygous line (¤) was used as a positive control, and negative control = no DNA (-).

The ability of Agrobacterium to transfer a DNA fragment to a plant cell provides a powerful tool for plant biotechnology. Transfered DNA (T-DNA) insertion within a plant genome has been confirmed to be a very effective way in studying gene function and regulation. Also, it can be used as a vector for the creation of transgenic plants.

Using PCR technique, the mutant analysis was done regarding the location of the T-DNA of *osm1*mutant. For, *osm1* mutant, the T-DNA was found to inserted in the promoter within a distance of 702 from the predicted translation start (ATG). This

result seems to be confirm the results of Sessions et al. in Ref.[26] which indicate the preference of T-DNA to be usually integrated outside the transcription regions. The exact site of the insertion can be of a distance from the expected one due to the overlapping reads of two or more sequences in the T-DNA lines or may be for other reasons [28].

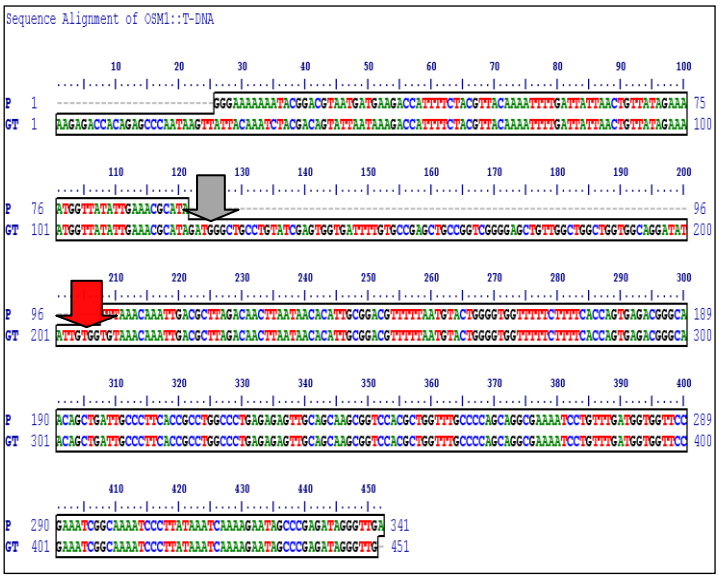

Figure 4: Sequence alignment of *osm1* homozygous shows the junction between the *OSM1* gene and the T-DNA using F_2R primers. Upper strand is the PCR product sequence (P). Lower strand is the published sequence in the database for the gene and the T-DNA (GT). The orange closed arrow indicates the correct left border of the insert and the gray closed arrow indicates the left border reported by the database. Sequence mismatch in the middle (----) indicates the missing part of the T-DNA.

Upon inserting T-DNA in plant's genome, a small deletion can occur whether in the plant genome or at the T-DNA termini [12, 15, 30]. For *osm1*mutant line tested in this research, a part of the T-DNA insertion was found to be deleted. Krysan et al. [11], Gheysen et al. [14], Mayerhofer et al. [19] and Zhu et al. [29] reported small deletion of the T-DNA at the left and the right borders. This usually happens during T-DNA integration.Generally, from 0-60bp has been said to be removed at

the left border of the T-DNA junction with the host sequence [12]. Since our investigation confirmed losing of 86bp, presumably, the sequence degradation during the integration process seems to be the reason for the nucleotides deletion[12].

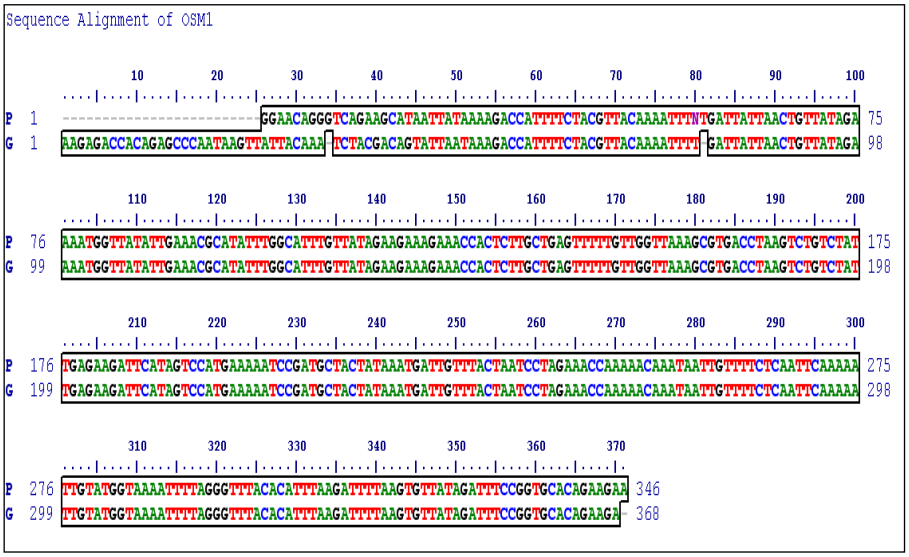

Figure 5: Sequence alignment of *OSM1* wild type showing the amplified fragment of *OSM1* gene using F_1R primers. Upper strand is the PCR product sequence (P), and the lower strand is the *OSM1* gene sequence published in the database (G).

In general, the majority of the mutants (88%) segregate in a Mendelian manner for the mutant phenotype and the segregation process may depend on the progeny and the number of inserts [13, 14]. When transformants contain one or two inserts, they usually show a clear Mendelian pattern of segregation. In case of more than four inserts the segregation process may differ as measured by the response to KanR,Kan^S and GUS^+ [14]. The reason for this could be explained by the deletion that occurs due to rearrangements during T-DNA integration [33] or due to the inactivation of foreign genes by methylation [18].

Upon genotyping selected population of *OSM1*, the segregation process seemed to be happening according to the Mendelian manner (1:2:1. Out of forty T_4 generation plants grown, 10 were wild type, 21 were heterozygous, and 9 were homozygous (Table 1). Chi-square analysis showed no significant difference from Mendelian ratio in *OSM1* genotype proportion. This pattern of segregation agrees with Feldmann's results in Ref. [12] who confirmed the normal and in addition to the exceptional phenotype ratio of T_3 generations.

Table 1: A Chi-square(X^2) test for *OSM1* genotype segregation; wild type (Wt), heterozygous (Het), and homozygous (Hom).

Type	population	proportion	Expected	Observed	df	X^2	P-value
Wt	40	0.25	10	10	2	0.15	0.928
Het		0.50	20	21			
Hom		0.25	10	9			

Drought tolerance:

Wilting symptoms and plant survival:

After exposing the plants to drought stress for 16 days, wilting symptoms appeared clearly on plants from both lines but homozygous plants were more sensitive to wilting than the wild type (Figure 6) and significant differences appeared between the two genotypes (Figure 7).Plant survival was taken after 11 days from plants recovering from drought stress. Both lines, homozygous and wild type plants showed no significant differences (Figure 8) but there was an indication of having better survival with the wild type plants. In a previous experiment on the response of this mutant to salt stress, *osm1* plants showed better survival than wild type plants even though there was a reduction in gene expression of OSM1 gene in the mutant comparing to the wild type plants. As salinity can lead to water deficit conditions

and affect plant water relations as a result of osmotic stress [5], I believe that the mutant plants under drought stress responded as if they were salt stressed. Together, our results demonstrate an important role of OSM1/SYP61 in osmotic stress tolerance which has a relation with the plant hormone ABA which plays a role in the regulation of stomatal responses in plants [30]. As my experiment was based on using a mutant carrying a T-DNA outside the coding region, a clear result would be collected upon using a mutant that carries T-DNA inside the gene where it interrupts the gene expression and prevents the formation of the specific protein.

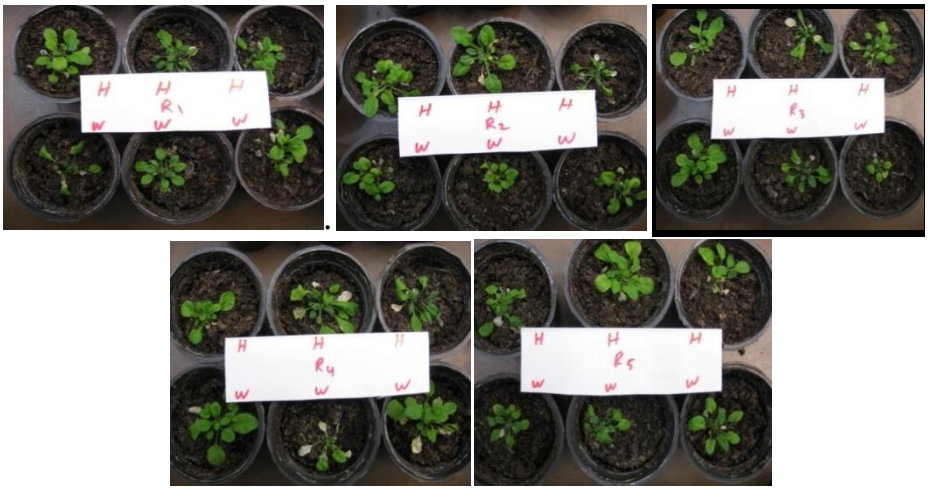

Figure 6. The wilting symptoms on homozygous and wild type plants of OSM1 gene after 16 days of exposure to drought stress. The mutant plants appeared more wilted than the wild type plants as statistically analysed (see figure 5).

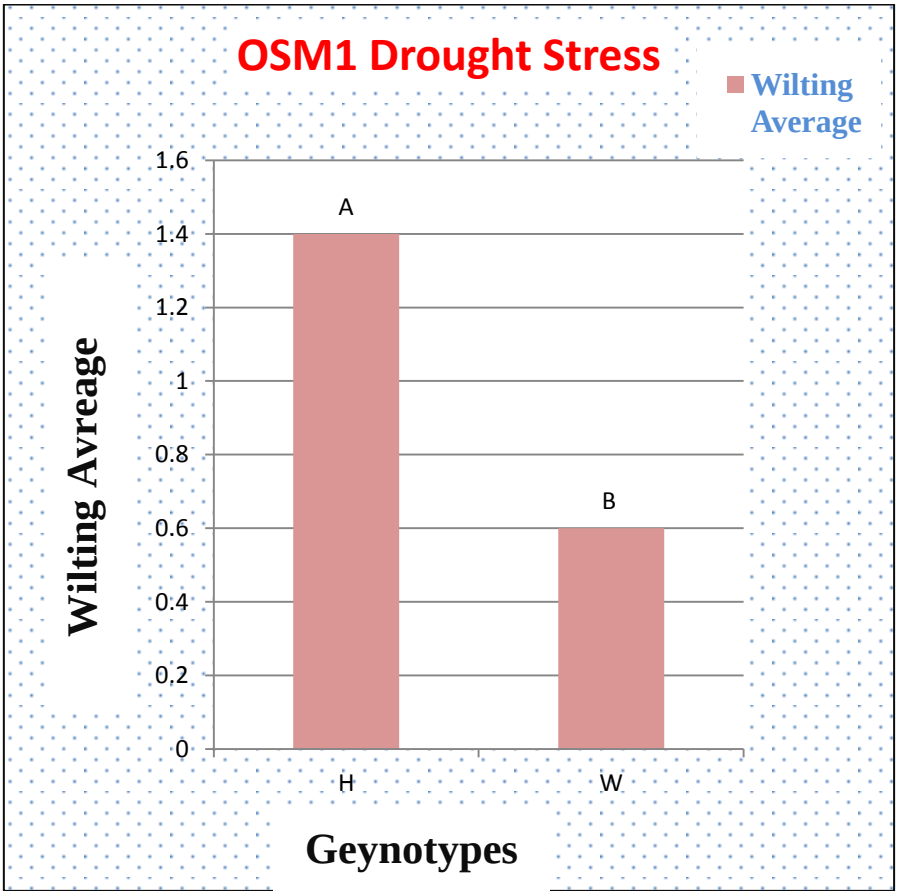

Figure 7: The effect of drought stress on wilting symptoms of homozygous (H) mutant and wild type (W) plants of OSM1 gene after 16 days of exposing to drought. One-way ANOVA analysis using Minitab 16 indicating significance between the two lines (*P-values*= 0.05).

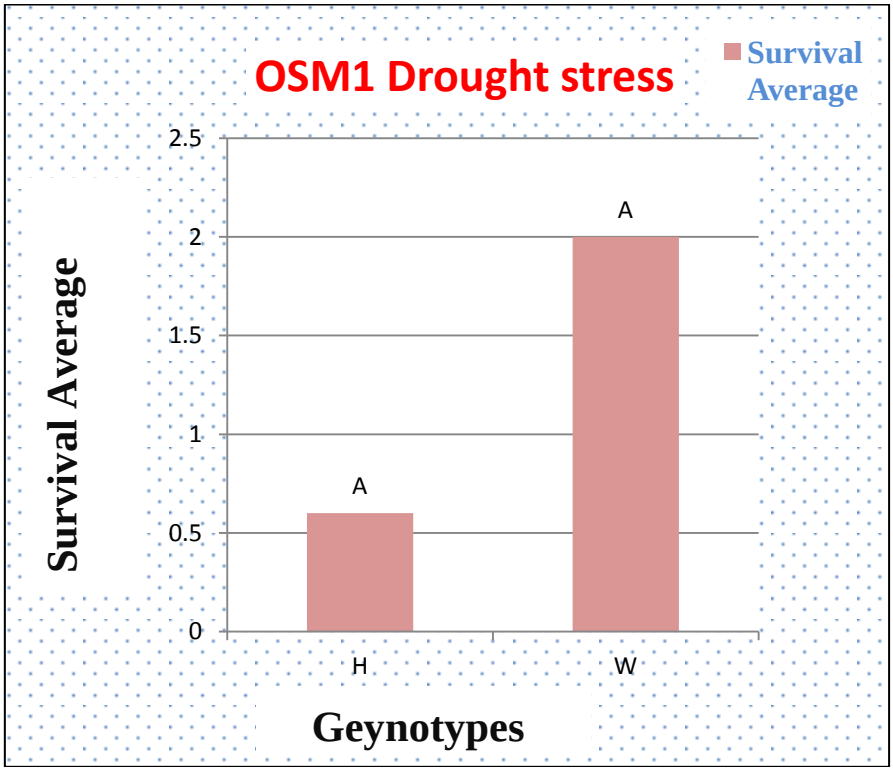

Figure 8: The effect of drought stress for 18 days on survival average of homozygous (H) mutant and wild type (W) plants of OSM1 gene after 11 days from recovering. One-way ANOVA analysis using Minitab 16 indicating no significance between the two lines (*P-values* = 0.098).

دراسة الجين SYP61/OSM1 في نبات الارابيدوبسيس تحت إجهاد الجفاف

أبوبكر علي الحاج *

المستخلص:

يعتبر الجفاف من الإجهادات البيئية اللاحيائية الرئيسة التي تؤدي إلى تدهور النمو الإنتاجية للمحاصيل الزراعية. تختلف النباتات طبيعيا، في حساسيتها للتأقلم للجفاف ودرجة تحملها له، وأكدت الأبحاث أن عدة جينات وعوامل نسخ تلعب دورا في استجابة النباتات لظروف نقص الماء. في هذه الدراسة تم اختيار الجين OSM1 (At1g28490) – والذي يشبه الجين SYP61– لدراسته لأول مرة تحت ظروف الجفاف للنباتات النامية في التربة. اشتمل البحث على دراسة نباتات الطفرة *osm1* (SALK_107167) مقارنة بالنباتات العادية المعرضة للجفاف. أكدت نتائج التعاقب القاعدي لنباتات الطفرة أن الـ T–DNA واقع في منطقة البروموتر للجين وهو الموقع الصحيح المحدد في قاعدة البيانات، غير أنه جزء بطول 86 زوجا قاعديا كان مفقودا عند الطرف الأيسر منه. بينت نتائج تحديد النمط الوراثي أن عملية الانعزال لنباتات الطفرة كانت وفق قواعد مندل للانعزال الوراثي. أوضحت نتائج النمط الظاهري أن نباتات الطفرة كانت أكثر حساسية لظهور أعراض الذبول مقارنة بالنباتات العادية، غير أن معدل البقاء لم يتأثر معنويا. هذه النتائج تدل على أن الجين OSM1 قد تكون له علاقة باستجابة نباتات الارابيدوبسيس لظروف نقص الماء، وهناك حاجة لدراسة مستوى التعبير الجيني له تحت هذه الظروف مع استخدام طفرة تحمل T–DNA في منطقة الجين.

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References:

1. Alonso J. M. and Stepanova A. N. (2003). T-DNA mutagenesis in Arabidopsis. Methods in molecular biology (Clifton, N.J.) 236 : 177-188.
2. Bowen R. (2000). Agarose gel electrophoresis of DNA. Available at: Boyer J. S. (1982) Plant productivity and environment.*Science*, 218:443-448
3. Boyes D.C., Zayed A.M., Ascenzi R., McCaskill A. J., Hoffman N. E., Davis K. R., and Gorlach J. (2001). Growth stage-based phenotypic analysis of Arabidopsis: A model for high throughout functional genomics in plants. Plant Cell 13: 1499-1510.
4. Bray E.A. (1997). Plant responses to water deficit. Trends in Plant Science 2: 48-54.
5. Chenna R., Sugawara H., Koike T., Lopez R., Gibson T.J., Higgins D. G., and Thompson J.D. (2003). Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res. 31: 3497-3500.
6. Edwards K., Johnstone C., and Thompson C. (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19 : 1349.
7. Elhaj A. (2009), Molecular and Physiological investigations of Arabidopsis mutant lines under abiotic stress. School of Biology, Newcastle University, Newcastle upon Tyne, UK. A PhD thesis.
8. Harb A., Krishnan A., Ambavaram M. M. R., and Pereira A. (2010). Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. Plant Physiol. 154: 1254-1271.
9. LeshemY., Golani Y., Kaye Y., and Levine A. (2010). Reduced expression of the v-SNAREs AtVAMP71/AtVAMP7C gene family in Arabidopsis reduces drought tolerance by suppression of abscisic acid-

dependent stomatal closure.Journal of Experimental Botany. 61 : 2615–2622.
10. Leung J.and Giraudat J. (1998). Abscisic acid and signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49: 199-222.
11. Krysan P.J., Young J.C., and Sussman M.R. (1999) T-DNA as an insertional mutagen in Arabidopsis. Plant Cell 11: 2283-2290.
12. Feldmann K. A. (1991). T-DNA insertion mutagenesis in Arabidopsis: Mutational spectrum. Plant Journal 1: 71-82.
13. Gandhi R., Matheshwari S.C. ,Khurana J., and Khurana P. (2001). Genetic and molecular analysis of *Arabidopsis thaliana* (ecotype 'Estland') transformed with *Agrobacterium vitro cell. Develop. Biol. Plant* .37: 629-637.
14. Gheysen G., Villarroel R., and Van Montagu M. (1991). Illegitimate recombination in plants: A model for T-DNA integration. Genes and Development 5: 287-297
15. Hall T.A. (2005). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. 41:95-98.
16. Higgins D. G. and Sharp P.M. (1988). CLUSTAL: A package for preforming multiple sequence alignment on a microcomputer. Gene 73: 237-244.
17. Hobbs S. L. A., Kpodar P., and Delong C.M.O. (1990) The effect of T-DNA copy number, position and methylation on reporter gene expression in tobacco transformants. Plant Mol. Biol. 15: 851-864.
18. Marris E. (2008). Water: more crop per drop. *Nature* 452: 273-277.
19. Mayerhofer R., Koncz-kalman Z., Nawrath C., Bakkeren G., Crameri A., Angelis K., Redei G. P., Schell J., Hohn B., and Koncz C. (1991) T-DNA integration: A mode of illegitimate recombination in plants. EMBO Journal 10: 697-704.

20. Neill S.J. and Burnett E.C. (1999) Regulation of gene expression during water deficit stress. Plant Growth Regulation 29 : 23-33.
21. Osmond C. B., Austin M.P., Berry J. A., Billings W.D., Boyer J. S., Dacey J.W. H., Nobel P. S., Smith S. D., and Winner W.E. (1987). Stress Physiology and the Distribution of Plants. BioScience37: 38-48.
22. Pan X., Liu H., Clarke J., Jones J., Bevan M., and Stein L. (2003). ATIDB: *Arabidopsis thaliana* insertion database. Nucleic Acids Research. 31: 1245-1251.
23. Radhamony R. N., Prasad A.M., and Srinivasan R. (2005) T-DNA insertional mutagenesis in Arabidopsis: A tool for functional genomics. Electronic Journal of Biotechnology 8: 82-106.
24. Sanderfoot A.A., Kovaleva, V., Bassham, D. C.,andRaikhle, N.V. (2001). Interactions between Syntaxins identify at least five SNARE complexes within the golgi/prevacuolar system of the Arabidopsis cell. Molecular Biology of the Cell 12:3733-43.
25. Sessions A., Burke E., Presting G., Aux G., McElver J., Patton D., Dietrich B., Ho P., Bacwaden J., Ko C., Clarke J.D., Cotton D., Bullis D., Snell J., Miguel T., Hutchison D., Kimmerly B., Mitzel T., Katagiri F., Glazebrook J., Law M., and Goff S. A. (2002). A high-throug out Arabidopsis reverse genetics system. Plant Cell 14: 2985-2994
26. Shao H. B., Chu L.Y., Jaleel C.A., and Zhao C.X. (2008). Water-deficit stress-induced anatomical changes in higher plants. Comptes Rendus – Biologies 331: 215-225.
27. SIGnAL (2000). Arabidopsis sequence indexed T-DNA insertion In http: 11 signal. Salk. Edu/ tdna-fa 95. Html.
28. Yang T., Zhang L., Zhang T., Zhang H., Xu S., and An L. (2005). Transcriptional regulation network of cold-responsive genes in higher plants. Plant Science 169 : 987-995.

29. Zhu J., Gong Z., Zhang C., Song C.P., Damsz B., Inan G., Koiwa H., Zhu J.K., Hasegawa P.M., and Bressan R.A. (2002). OSM1/SYP61: A syntaxin protein in Arabidopsis controls abscisic acid-mediated and non-abscisic acid-mediated responses to abiotic stress. Plant Cell 14 : 3009-3028.
30. The Arabidopsis Genome Initiative (TAGI) (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408 : 796-815.
31. QIAGEN (2002) QIAQUICK Spin Handbook available at:
32. Van der GRAAFF E., and Hooykaas P. J. J. (1996) Improvements in the transformation of Arabidopsis thaliana C24 leaf-discs by *Agrobacteriumtumefaciens*. Plant Cell Rep. 15 : 572-577.